ALTHOUGH myelofibrosis with myeloid metaplasia (MMM) was first described nearly one hundred years ago, the etiology of this disturbance remains uncertain. Two interpretations have been offered: first, that there is a primary insult to bone marrow resulting in necrosis and necrobiosis followed by a reparative stage of fibrosis of marrow and compensatory extramedullary hematopoeisis in the liver and spleen. The second interpretation suggests that MMM is a generalized proliferation of mesenchyme with differentiation to fibroblasts and their end product, collagen, in the bone marrow and to hematopoietic tissue in the liver and spleen. According to this view, MMM is pictured as a neoplastic proliferative disturbance related to polycythemia vera, acute and chronic myelogenous leukemia, primary thrombocythemia and Di Guglielmo's disease—all components of the myeloproliferative syndrome (MPS). The fact that these apparently disparate conditions may be difficult to distinguish from one another, and with varying frequencies transmute into one another, has been an important argument in formulating the neoplastic hypothesis. Although the latter concept for the etiology of MMM is currently the most widely accepted, there is little experimental evidence to indicate whether or not either of these hypotheses are correct. In the present paper, we describe the production of a form of MMM by the intravenous injection in rabbits of saponin.

MATERIALS AND METHODS

Two kg. male New Zealand white rabbits were used throughout the study. They were housed individually, fed commercial rabbit chow and given tap water for drinking. Saponin (Mann) freshly dissolved in physiologic saline was injected into the lateral ear vein at a dose of 1.2 mg./kg. every fourth day for a period of three weeks. Control animals received intravenous saline rather than saponin. Sequential blood studies including hematocrit, platelet count, reticulocyte count, differential and enumeration of nucleated red cells were performed by routine technics. Both groups of animals were killed after three weeks and autopsied. Specimens of pelvic bone marrow, liver and spleen were fixed in 10 per cent formalin and stained with hematoxylin and eosin. Bone marrow was, in addition, stained with Masson's trichrome for clear demonstration of fibrous tissue.
to fixation, the spleens were weighed, cut, and touch imprints made that were stained with Wright-Giemsa. All histologic and cytologic materials were examined by two observers who were unaware of the experimental status of the animals. No attempt was made to grade the degree of myelofibrosis or myeloid metaplasia since such lesions were not seen in any of the control animals.

**Results**

Two (2) of 19 saponin injected animals expired during the study. Hematologic and histologic data were available for 17 animals injected with saponin and an equal number of controls. A decrease in the hematocrit of approximately two units was uniformly observed by the end of the course of saponin treatment. White count, differential and platelet counts were essentially unchanged throughout the study. Within one day following the administration of saponin, an intense normoblastemia developed with the total number of such cells often constituting as much as 50 per cent of the nucleated cell count of the peripheral blood. After seven days, the number of nucleated cells diminished but increased numbers were still present when the animals were killed on the twenty-first day. Reticulocyte counts tended to rise considerably although not to the high levels as did the normoblasts. In general, the reticulocytosis paralleled the nucleated red count. Although poikilocytosis was not striking, tear-drop cells were noted in the peripheral smears following saponin administration.

The splenic weight in the controls had a mean value of 1.6 grams. The weight of this organ in the experimental group was quite variable, but the average weight (10 grams) considerably exceeded that observed in controls.

Twelve (12) of the 17 saponin treated animals developed myelofibrosis (Fig. 1). Fibrosis was often quite marked and evident by hematoxylin and eosin but the presence of collagen was always confirmed by demonstrating its tinctorial properties with a trichrome stain. The degree of persistent marrow cellularity was variable; hypercellularity often coexisted with severe fibrotic lesions. No attempt was made to quantitate the number of megakaryocytes in a given field, but like other hematopoietic cells of the marrow, these were frequently seen in the same field as the fibrosis. Ten (10) of the 17 animals showed myeloid metaplasia of the liver (Fig. 2 and 3), 12 of the spleen (Fig. 4). Although the most easily recognizable feature was the megakaryocytosis, erythrocytic and granulocytic precursors, normally found only in the bone marrow, could readily be observed. Such hematopoietic tissues were more readily identified and classified in touch preparations of the spleen than in formalin-fixed biopsies.

A summary of the histologic data for the experimental group is shown in Table 1.

No indications of either myelofibrosis or myeloid metaplasia were observed in any of the control animals.

**Discussion**

These results indicate that morphologic lesions similar to those of human MMM are readily induced by the intravenous administration of the hydro-
Fig. 1.—Photomicrograph of section of bone marrow showing fibrosis and cellular areas containing megakaryocytes and other hematopoietic elements. Hematoxylin & Eosin ×60.

carbon, saponin. A possible dose relationship for the production of these lesions was not established since tissues were examined only after three weeks of treatment. The marked normoblastemia that followed the first injection, however, suggests that alterations might be induced by a single dose. Additional studies including serial biopsies will be required before one can determine the temporal events involved in the full expression of these lesions.
Fig. 2.—Photomicrograph of section of liver showing extramedullary hematopoiesis with three (3) megakaryocytes in field. Hematoxylin & Eosin ×150.

Although it is tempting to draw analogies to human MMM, a great deal more must be learned concerning the natural history of the saponin induced lesions. Do the lesions regress ultimately, or is there progression into chronicity, or possibly, to a blastic crisis?6

Earlier in this century, saponin was utilized by Bunting for the production of hemolytic anemia.7 Normoblastemia and the appearance of megakaryocytes in the liver and spleen were observed, but only a few rabbits were studied and no parallels to MMM in man drawn. Firket and Campos subsequently
reported that marked thrombocytopenia followed the administration of saponin and confirmed that megakaryocytes appeared in organs. These observers suggested that a generalized megakaryocytic response had resulted from saponin poisoning. In the dosages of saponin used in our studies neither significant anemia nor thrombocytopenia developed.

Aromatic hydrocarbons as exemplified by methylcholanthrene and 7, 12-dimethylbenz(a)anthracene have been shown to induce malignant neoplasias. The similar ring structure of saponin may, therefore, endow it with
carcinogenic properties and suggest that what has been induced in the present experiment is a neoplastic disturbance akin to the leukemias that have been produced in animals by such agents.

Hunstein et al. recently reported producing myelofibrosis with myeloid metaplasia by injecting rabbits with guinea pig anti-rabbit bone marrow serum. Similarities between these two apparently different technics for induction of MMM must await further investigation.

Although clinically, MMM occurs most often as an isolated syndrome, i.e., agnogenic myeloid metaplasia, it has also been reported in association with tuberculosis, solid tumors, after exposure to industrial toxins, and following nuclear radiation. It should therefore be realized that a variety of etiologic agents may either quickly or slowly eventuate in myelofibrosis with myeloid metaplasia and that the chronic disease bearing this name may have but little relationship to more acute forms. Thus, the relevance of the present experimental model to the morphologically similar chronic disease in man is at present obscure.
MYELOFIBROSIS AND MYELOID METAPLASIA

Table 1.

<table>
<thead>
<tr>
<th>Animal</th>
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Positive/Total: 12/17 (71%) 10/17 (59%) 12/17 (71%)

SUMMARY

A simple and reproducible model for inducing a form of myelofibrosis with myeloid metaplasia by the injection of saponin intravenously is described. The histologic lesions produced resembled those of agnogenic MMM in man.

SUMMARIO IN INTERLINGUA

Es describite un simple reproducibile modello pro le induction de un forma de myelofibrosis con metaplasia myeloide utilisante intravenose injectiones de saponina. Le lesiones histologic assi produce resimilava le correspondente lesiones de agnogenic myelofibrosis con metaplasia myeloide in humanos.

REFERENCES

4. Hutt, M. S. R., Pinninger, J. L., and
Experimental Induction of Myelofibrosis with Myeloid Metaplasia

SALVATORE A. P. ARGANO, MARK S. TOBIN and DAVID M. SPAIN

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